REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

The November 18, 2009, personal interview between SPE Low, Examiner Liu, applicant Francis Barany, and applicants' undersigned attorney is gratefully acknowledged. The substance of the interview is summarized below.

Claims 89–110, 112, and 149–153 are pending. Claims 89, 90, and 149–152 have been amended. No new matter has been added.

The United States Patent and Trademark Office ("PTO") has taken the position that claims 89–97, 109, 112, and 149–153 are not entitled to the priority date of U.S. Provisional Application Serial No. 60/011,359 ("'359 application"), filed February 9, 1996, because the recitation that each capture oligonucleotide at an array position has "greater than sixteen nucleotides" and "differs in sequence from adjacent capture oligonucleotides, when aligned to each other, by at least 25% of the nucleotides" is not supported in the '359 application. Applicants respectfully disagree.

The disclosure of the '359 application fully supports the above cited claim limitations. Support for the limitation that the capture oligonucleotides of the array have greater than sixteen nucleotides is found on page 7, lines 31–33 and original claim 120 of the '359 application. Further, the recitation that each capture oligonucleotide differs in sequence from adjacent capture oligonucleotides, when aligned to each other, by at least 25% of the nucleotides is also fully supported by the disclosure of the '359 application at page 45, lines 32–34 and original claims 81 and 111.

Therefore, because the '359 application more than adequately supports the limitations of claims 89–97, 109, 112, and 149–153, applicants respectfully submit that these claims are entitled to a priority date of February 9, 1996.

The objection to claim 89 for missing a period is respectfully traversed in view of the amendment above.

The rejection of claims 89–97, 109, 111, 112, and 148–153 under 35 U.S.C. § 112 (second para.) for indefiniteness is respectfully traversed in view of the above amendments except to the extent set forth below.

The PTO contends that it is not clear if the capture oligonucleotides or the multimer nucleotides have "greater than sixteen nucleotides." Applicants disagree. In claim 89, which recites ". . . the nucleotide multimers are selected so that each of the plurality of

capture oligonucleotides, formed from a plurality of assembled nucleotide multimers and attached to the solid support at each array position, have greater than sixteen nucleotides . . .", it is clear that the capture oligonucleotides each have greater than sixteen nucleotides.

In view of the forgoing, applicants respectfully request that the rejection of claims 89–97, 109, 111, 112, and 148–153 for indefiniteness be withdrawn.

The rejection of claims 89–97, 109, 111–112, and 148–153 under 35 U.S.C. § 103 for obviousness over U.S. Patent No. 5,510,270 to Fodor et al. ("Fodor") in view of U.S. Patent No. 5,474,796 to Brennan et al. ("Brennan") and U.S. Patent No. 5,594,121 to Froehler et al. ("Froehler") is respectfully traversed for the reasons set forth in the accompanying Declaration of Francis Barany, Ph.D., under 37 C.F.R. § 1.132 ("Barany Declaration").

Prior to the filing date of the above referenced patent application, it was well appreciated in the art that the ability to accurately identify low abundance nucleic acid sequence variations, including single nucleotide polymorphisms, insertions, deletions, or translocations at multiple adjacent, nearby, and distant genomic loci would have profound implications for the identification of genetic disorders, the diagnosis and treatment of cancer, and the detection of infectious diseases (Barany Declaration at ¶ 5). Cancer, for example, can arise from the accumulation of mutations in genes controlling cell cycle, apoptosis, and genome integrity (*id.*). Oncogenes may be activated by point mutations, translocations, or gene amplification, while tumor suppressor genes may be inactivated by point mutations, frameshift mutation and deletions (*id.*). These mutations may be inherited or somatic, arising from exposure to environmental factors or from malfunctions in DNA replication and repair machinery (*id.*). Since the capacity to detect these cancer related mutations would significantly enhance cancer detection and diagnosis, and identify the most effective and targeted cancer treatment protocols, there was a well recognized need in the art for an assay that could achieve early and accurate detection of these cancer related mutations (*id.*). The efforts of others in the art to develop a hybridization array-based detection assay to meet this need have failed and continue to fail (*id.*). However, the array with capture oligonucleotides of the present invention having greater than sixteen nucleotides and sequences selected to hybridize with complementary oligonucleotide target sequences under uniform hybridization conditions across the array of oligonucleotides with minimal cross reactivity, where each capture oligonucleotide of the array differs in sequence from other adjacent capture oligonucleotides, when aligned to each other, by at least 25% of the nucleotides (hereafter

identified as "Applicants' Array Design"), has overcome these failures and has successfully resolved this unmet and long-felt need (*id.*).

The development of an assay suitable for the detection of cancer related mutations was fraught with challenges (*id.* at ¶ 6). The first challenge was to identify an approach that could detect very low abundant target mutations within a patient sample containing a plurality of closely related non-target sequences (*i.e.*, normal, non-mutant sequence) (*id.*). In primary tumors for example, normal stromal cell contamination can be as high as 70% of total cells (*id.*). Therefore, a mutation present in only one of the two chromosomes of a tumor cell may represent as little as 15% of the DNA sequence in a sample (*id.*). In addition, early detection of such mutations requires the ability to detect as few as one mutant copy of a nucleic acid sequence in the presence of over 100 non-mutant copies of the nucleic acid sequence (*id.*). Accordingly, the detection assay had to be highly sensitive (*id.*). A second challenge was to develop a highly specific assay having the capacity to reproducibly discriminate and detect a plurality of often closely spaced mutations in multiple genes without generating false-positive or false-negative results (*id.*). Finally, it was highly desirable to employ an assay that could achieve this highly sensitive and specific mutation detection in non-invasively collected patient samples to help reduce overall cost and, more importantly, alleviate patient discomfort (*id.*).

Although the advent of DNA array technology, based on direct hybridization of target sequence to the array, resulted in a paradigm shift in identifying expression changes and known SNPs on a genomic scale, it failed, and, as discussed below, continues to fail to meet the above noted challenges in detecting mutations (*id.* at ¶ 7).

Typical DNA hybridization arrays are designed to simultaneously discriminate and detect multiple target sequences differing in sequence by only one or a few nucleotides (*id.* at ¶ 8). Target sequence discrimination using a hybridization array depends on the highly specific binding affinity of the immobilized capture oligonucleotides to their complementary labeled target sequences (*id.*). The hybridized labeled target sequences are subsequently detected and identified by their location of hybridization on the array surface (*id.*).

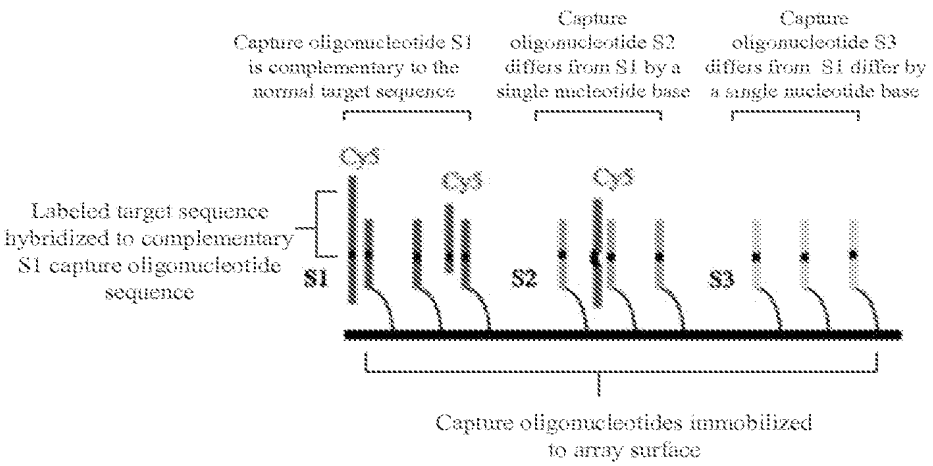

Figure 1: A typical direct hybridization array having capture oligonucleotides designed to simultaneously discriminate and detect multiple nucleotide variations at multiple adjacent and nearby genetic loci.

Figure 1 depicts a typical hybridization array having capture oligonucleotide probes immobilized on the array surface where the capture probes are designed to discriminate nucleotide variations (*i.e.*, allelic variations) at multiple adjacent and nearby loci (*e.g.*, S1, S2, and S3) (*id.* at ¶ 9). The capture oligonucleotides responsible for allelic discrimination at a particular locus (*e.g.*, S1, S2, or S3 in Figure 1) differ from each other by only a single nucleotide base substitution, insertion, or deletion, and, therefore, have very similar nucleotide sequences as shown in Figure 2 below (*id.*). Consequently, these capture probes also have very similar melting temperatures (Tm) (*id.*).

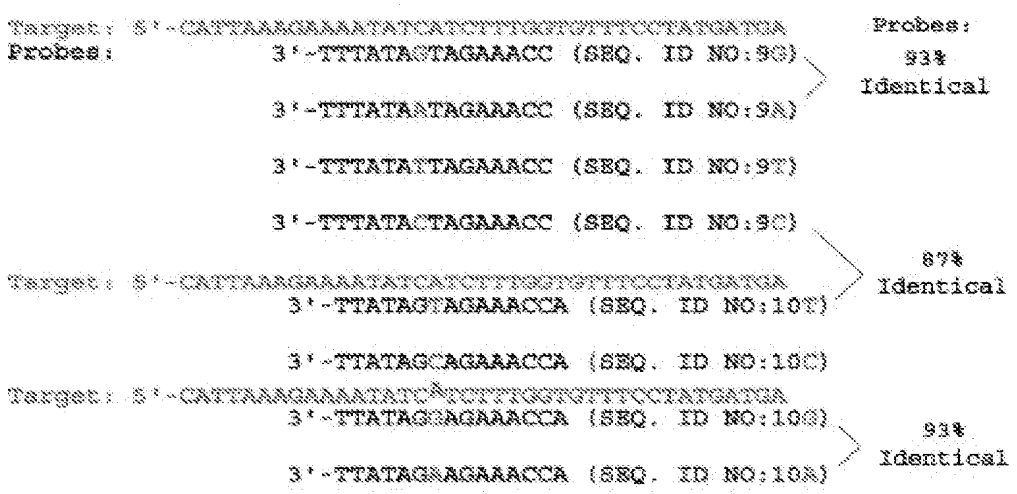

Figure 2: Capture oligonucleotide probes of a typical hybridization array. Figure 2A shows that capture oligonucleotide probes designed to detect target sequence variations at one nucleotide position share 93% sequence identity while probes designed to detect target sequence variations at nearby genetic loci (i.e., probes of 2A and 2B) share 87% sequence similarity.

In addition to capture probes designed to detect sequence variations at a single locus or nearby loci, typical hybridization arrays also contain capture probes that are designed to detect distal mutations (*id.* at ¶ 10). Because capture probes are target-sequence specific, the nucleotide sequences of probes detecting distal target sequences will differ significantly in both sequence and melting temperature from other capture probes on the array designed to detect other distant mutations (*id.*).

Figure 3 below illustrates some of the problematic results that emanate from the typical direct hybridization array designed to detect target sequences having overlapping sequence homology (*e.g.*, target sequences that have only single nucleotide differences) (*id.* at ¶ 11). It is possible for a single target to bind to multiple oligonucleotide probes with different, yet similar sequences due to mismatched cross hybridization (*id.*). Cross hybridization will result in the generation of false-positive signals (*id.*).

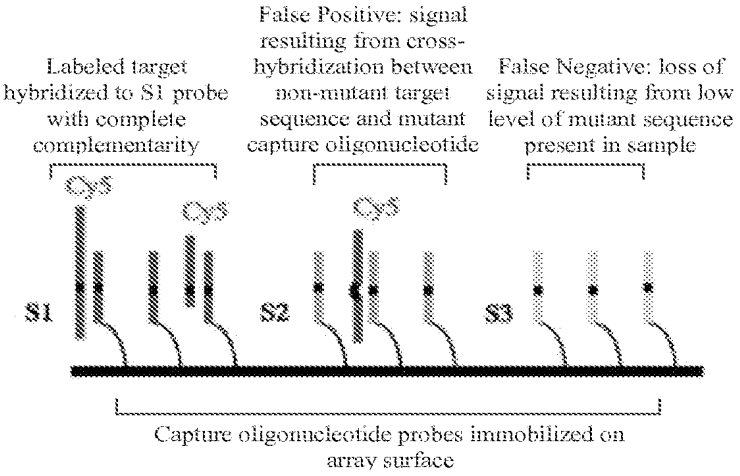

Figure 3. Problematic results of a typical hybridization array

Another major drawback of hybridization arrays is their propensity to generate false-negative signals (*id.* at ¶ 12). As noted above, significant variability in nucleotide sequence and melting temperature exists between capture probes that are designed to detect distant mutations (*id.*). Since optimal hybridization conditions for a target and its complementary capture probe are sequence specific, employing uniform, highly stringent hybridization conditions across the array that are suitable for all capture probe-target sequence pairs is difficult, if not impossible (*id.*). The application of hybridization conditions that are overly stringent for some sequences will prevent target-probe hybridization and lead to the loss of signal (*i.e.*, false-negative signal) (*id.*). Since the melting temperature between

target and probe sequence varies across the array, stringent hybridization conditions will result in weak or missing signal from low abundant mutations (*id.*). If non-stringent conditions are used to detect low level mutations, this will significantly increase the likelihood of cross-hybridization leading to false-positives (*id.*).

In addition to the possibility that a single target will bind to multiple oligonucleotide probes with different, yet similar sequences due to mismatched cross hybridization, there are many other competitive processes that influence signal intensity values generated during array hybridization (*id.* at ¶ 13). Besides the desired target binding to probe (Figure 4A), there is the undesired probe self binding (Figure 4B), folding of target to reduce binding to probe (Figure 4C), and dimerization of adjacent probes (Figure 4D) as illustrated below (*id.*).

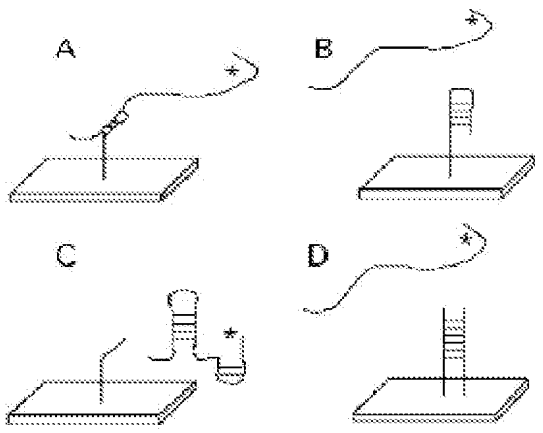

Figure 4: Depiction of four competitive processes on signal intensity values. Each panel shows a labeled (*) target and an immobilized probe on a microarray. (**A**) hybridization of a target to a probe; (**B**) probe self-folding; (**C**) folding of the target and (**D**) dimerization of adjacent probes (reproduced from Pozhitkov et al., *Nucleic Acids Research* 34(9):e66 (2006))

While a great deal of effort has been invested in developing design strategies that generate capture probes having minimal cross-reactivity to non-complementary target sequence, minimal cross-reactivity to other probe sequences, and do not undergo self-folding, none of these efforts have proven successful (*id.* at ¶ 14). These design strategies, typically based on the thermodynamic properties of the capture oligonucleotides (*e.g.*, guanine-cytosine content, secondary structure, melting temperature, etc.), attempted to predict oligonucleotide duplex formation (*id.*). Using these strategies, capture probes were designed so that a single mismatch base pair would, theoretically, significantly lower the binding affinity of the mismatched duplex compared to the corresponding perfectly matched duplex at a given temperature (*id.*). These differential binding affinities of a target sequence to a

mismatch or perfect match probe provide the basis of sequence discrimination, allowing for the identification of target sequence because it is bound to a perfect match but not a mismatched probe at a specified temperature (*id.*).

Figure 5 below depicts the melting curve profiles, *i.e.*, the temperature-dependent dissociation of capture probe bound to its target sequence, for a set of mismatch and perfect match probes (*id.* at ¶ 15). Ideally, the melting profiles of the mismatch probe and perfect match probe would be tight to facilitate unambiguous differential signal detection (*id.*). However, as indicated by the red arrow in the top panel, some mismatched probes have melting curves that overlap exactly with the perfect matched probes, making it impossible to distinguish target binding to the mismatched probe from the perfect match probes (*id.*). The first derivatives of these melting curves shown in the bottom panel of Figure 5, more clearly illustrates the overlap in melting temperatures between the mismatch and perfect match probes (red arrow) (*id.*). In addition, it shows that some mismatched probes produce signal over a broad temperature range, generating signal that overlaps with both mismatch and perfect match probes (*see* bottom panel of Figure 5) (*id.*). These mismatch probes also cannot be accurately distinguished from perfect match probes (*id.*).

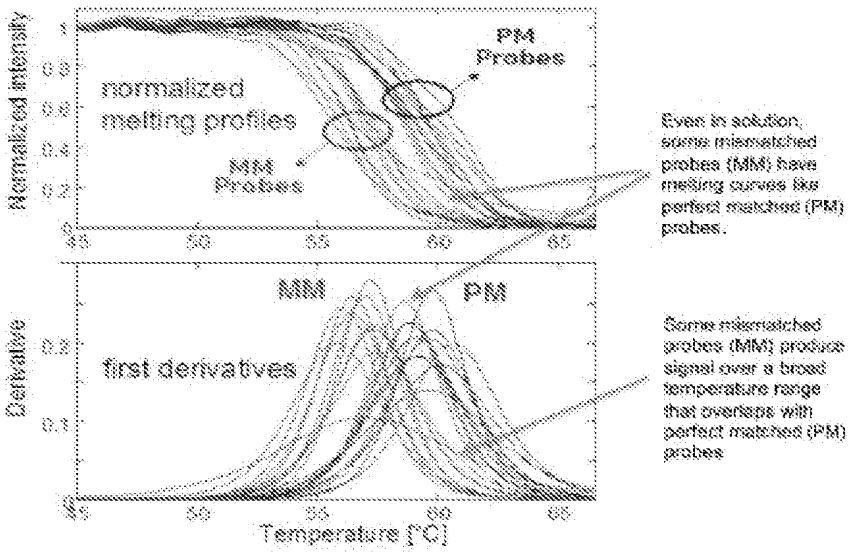

Figure 5: Melting profiles of mismatch (MM) and perfect match (PM) oligonucleotides probes in solution (reproduced from www.genewave.com/images/manager/hyblive_schema1.jpg). The top panel is a plot monitoring the decrease in signal intensity that occurs as the DNA duplex melts with increasing temperature. The bottom panel is a plot of the negative first derivative of the change in fluorescence (-dF/dT, the rate of change of fluorescence) versus temperature. The distinct peaks in this plot correspond to the melting temperature of each DNA duplex.

Naiser et al., "Impact of Point-Mutations on the Hybridization Affinity of Surface-Bound DNA/DNA and RNA/DNA Oligonucleotide-Duplexes: Comparison of Single Base Mismatches and Base Bulges," *BMC Biotech.* 8:48 (2008) ("Naiser") describes a comprehensive analysis of how single nucleotide variations (referred to as "point defects") affect the hybridization of fluorescently labeled oligonucleotide targets to surface-bound oligonucleotide probes (*id.* at ¶ 16). Naiser generated a set of point mutated probes derived from a common probe sequence motif that was complementary to a region of a target sequence (*id.*). Exemplary probe sequences representing single nucleotide substitutions, insertions, and deletions at the first two bases (*i.e.*, first two defect positions) of the probe sequence are depicted in Figure 1 of Naiser (reproduced below as Figure 6) (*id.*).

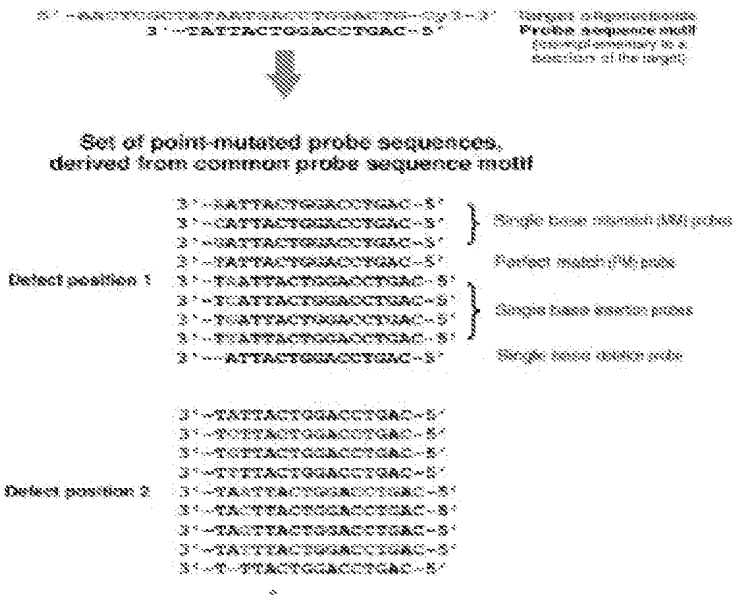

Figure 6: Figure 1 of Naiser et al., *BMC Biotech.* 8:48 (2008) showing the comprehensive set of point-mutated probes is derived from a common probe sequence motif which is complementary to the target sequence. Probe sequences are shown for the first two defect positions only.

The probe sequences were arranged on a microarray surface as a compact feature block and a sample containing a single target nucleotide sequence was contacted with the array surface to facilitate target-probe hybridization (*id.* at ¶ 17). Hybridization signals resulting from target sequence hybridization to individual probes in the probe set were plotted against the position of the defect in the probe sequence to create a defect profile (*id.*). The defect profile shown in Figure 7 (below) provides a direct comparison of the binding affinities for a plurality of mismatch oligonucleotide duplexes (*i.e.*, duplexes between target and probe where the probes differ from the target sequence by a single base mismatch,

insertion, or deletion), and demonstrate the considerable variability in binding affinities that exist between mismatched probe sequences (*id.*).

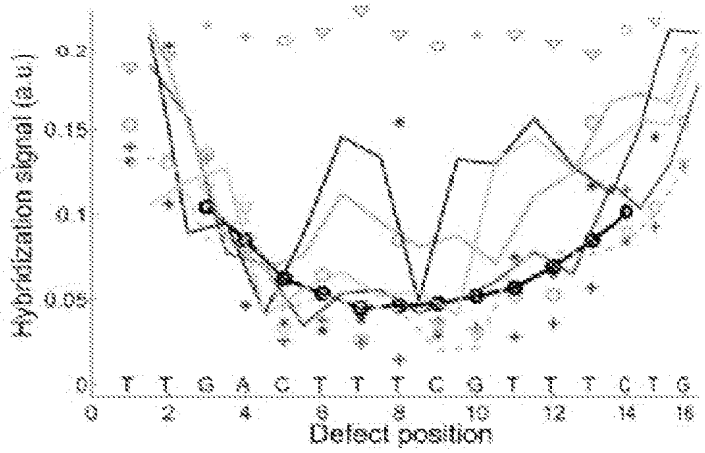

Figure 7: Figure 6 of Naiser et al., *BMC Biotech.* 8:48 (2008) showing the direct comparison of single base mismatches, insertions and deletions. The 16 mer probe sequence motif 3'-TTGACTTTCGTTTCTG-5' is complementary to the target BEI. Hybridization signals (data processing: raw fluorescence intensities; solution-background correction) of single base mismatch probes with substituent bases A (red crosses), C (green circles), G (blue stars), T (cyan triangles), running average of mismatch intensities (black line); perfect match probe signals (grey symbols) single base insertion probes (solid lines) with insertion bases A (red), C (green), G (blue), T (cyan). Hybridization signals of single base deletions (orange dashed line) are comparable to that of mismatches at the same position. Increased hybridization signals of certain insertion defects are due to positional degeneracy of base bulges

The defect profiles of Naiser reveal that the dominant parameter determining oligonucleotide probe-target affinity – on the microarray surface – is the position of the defect (*id.* at ¶ 18). The grey symbols in the defect plot represent the intensity signal generated by perfect match probe binding to its complementary sequence (*id.*). A moving average of the hybridization signal across defect positions reveals a trough-like "mean profile" curve (represented by a solid black line in the defect plots) that provides a reasonable approximation for the average position dependence obtained from a large number of different sequence motifs (*id.*). For 16mer duplexes, for example, a single base mismatch in the center of the duplex typically results in 25% of the perfect match (PM) hybridization signal while a mismatch near or at the end of the duplex results in 50% to 75% of the PM hybridization signal (*id.*). However, for individual sequence motifs, significant sequence-dependent deviations from the simple position dependence were also observed (*id.*). For example, a single base (G) insertion between positions 6–7 and 10–12 generates a mismatch signal intensity that is approximately 75% of the perfect match signal (*id.*). Unexpectedly, a single base substitution at the central position 8 (T→G) generates a mismatch signal intensity that approaches 75% of the perfect match signal (*id.*). This defect plot clearly illustrates the

unpredictable influence that a single nucleotide variation can have on probe-target binding affinity (*id.*).

Figure 8 below is a fluorescence micrograph of the microarray feature-block illustrating the problem associated with trying to accurately discriminate mismatch and perfect match probe binding to target sequence based on hybridization signal intensity (*id.* at ¶ 19). The microarray feature-block comprises variations of a 16mer probe sequence motif (*i.e.*, probe sequences varying by single base insertions, deletions, and substitutions) subject to hybridization to a single nucleic acid target sequence (*id.*). Each 3 x 3 sub-array comprises one perfect matching probe, three single base mismatch probes, four insertion probes, and one single base deletion probe (*id.*). While the signal intensity generated by the perfect match probe is distinguishable from the signal intensity generated by the mismatch probes in the center two sub-arrays outlined in red (*i.e.*, the single brightest square within each sub-array represents the intensity of the perfect match probe), there is still considerable signal observed at other positions (*id.*). Further, where mismatches are elsewhere, it is even more difficult to distinguish signal intensities generated by perfect match and mismatch probes (*id.*).

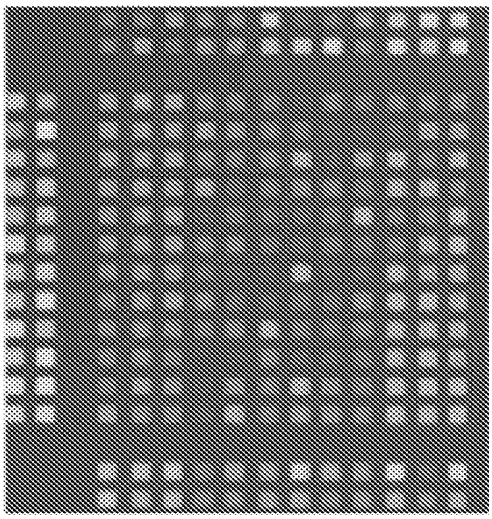

Figure 8: Fluorescence micrograph of a microarray feature-block comprising variations of the 16 mer probe sequence motif 3'-TATTACTGGACCTGAC-5'. Microarray hybridization was performed with the 5'-Cy3-labeled RNA oligonucleotide target 3'-AACUCGCUAUAAUGACCUGGACUG-5' (target concentration: 1 nM in 5 × SSPE, pH 7.4, 0.01% Tween-20, T = 30°C). Each 3 × 3 sub-array comprises one perfect matching probe, three single base mismatch probes, four insertion probes and one single base deletion probe. Figure reproduced from Naiser et al., "Position Dependent Mismatch Discrimination of DNA Microarrays – Experiments and Model," *BMC Bioinformatics* 9:509 (2008) (attached hereto as Exhibit 2).

Naiser's findings are consistent with those reported in an earlier study by Pozhitkov et al., "Test of rRNA Hybridization to Microarrays Suggest that Hybridization Characteristics of Oligonucleotide Probes for Species Discrimination Cannot be Predicted,"

Nucleic Acids Research 34(9):e66 (2006) ("Pozhitkov") (*id.* at ¶ 20). Pozhitkov assessed the utility of *in silico* predictions of probe-target duplex stabilities using DNA arrays for the detection of rRNA sequences (*id.*).

Pozhitkov's assessment of the effects of mismatches in the probe sequence on signal intensity values generated when hybridized to the non-mismatched target sequence also revealed that mismatch position, mismatch type, and the type of neighboring nucleotides surrounding the defect have significant effects on the normalized signal intensity values *(id.* at ¶ 21). "Moving the MM base away from the 5' or 3' termini to the center of the probe significantly decreases signal intensities (Figure 3). … However, we emphasize that this was an average result, and note that in some individual cases, MM probes with central mismatches (positions 9–11) were observed to have signal intensities that were *equal to or 1.6 times higher* than the corresponding perfectly matched probe" (*id.*).

The implications of Pozhitkov's and Naiser's findings are that direct hybridization methods which attempt to simultaneously discriminate and detect nucleic acid sequence variations are inadequate because of the unpredictable cross-hybridization between target sequence and mismatch or perfect match probe sequences (*id.* at ¶ 22). Thermodynamic parameters are simply not capable of accurately predicting mismatch and perfect match oligonucleotide duplex formation (*id.*). Accordingly, despite tremendous efforts to improve the reliability and reproducibility of the technology, the findings of Naiser and Pozhitkov described above, clearly indicate that a fundamental understanding of the technology is lacking and that even current approaches for microarray design are inadequate (*id.*). Likewise, this same problem extends to any assay format where the target sequence is detected and distinguished from other sequences by its hybridization to a complementary sequence (*id.*).

Fodor relates to a method for synthesizing and screening polymers on a solid substrate (*id.* at ¶ 23). The method involves providing a substrate which may include linker molecules on its surface (*id.*). On the substrate or a distal end of the linker molecules, a functional group with a protective group is provided (*id.*). The protective group may be removed upon exposure to radiation, electric fields, electric currents, or other activators to expose the functional group (*id.*). Concurrently or after exposure of a known region of the substrate to light, the surface is contacted with a first monomer unit M1 which reacts with the functional group which has been exposed by the deprotection step (*id.*). Thereafter, second regions of the surface (which may include the first region) are exposed to light and contacted

with a second monomer M2 (which may or may not be the same as M1) having a protective group (*id.*). These steps are repeated until the substrate includes desired polymers of desired lengths (*id.*). Monomers may include amino acids, nucleotides, pentoses, and hexoses (*id.*).

Fodor does not teach arrays of oligonucleotides on a solid support where each oligonucleotide of the array differs in sequence from other adjacent oligonucleotides when aligned to each other by at least 25% of the nucleotides (Barany Declaration at ¶ 24).

Further, the PTO acknowledges that Fodor does not teach selecting nucleotide multimers where a selected nucleotide multimer has a nucleotide sequence that differs from the nucleotide sequence of another selected nucleotide multimer by at least two nucleotides and where no two dimers forming a nucleotide multimer are complementary to each other. In addition, Fodor fails to suggest that the selected nucleotide multimers do not result in self-pairing or hairpin formation.

Brennan relates to an apparatus and methods for making arrays having functionalized binding sites on a support surface and conducting a large number of chemical reactions on the support surface (*id.* at ¶ 25). Brennan further relates to a method of determining or confirming the nucleotide sequence of a target nucleic acid where the target nucleic acid is labeled and hybridized to oligonucleotides of known sequence bound to sites on the array plate (*id.*).

It is applicants' understanding that the PTO considers Brennan's disclosure of arrays having 3-mers and 10-mers attached thereto where every possible permutation of the 3-mer or 10-mer is provided, to be the same as each capture oligonucleotide of an array differing in sequence from other adjacent capture oligonucleotides when aligned to each other by at least 25% (*id.* at ¶ 26). Applicants respectfully disagree for the reasons set forth below (*id.*).

Although Brennan teaches that the resulting 10-mer oligonucleotides on an array represent all permutations of the 10-mer sequence, each 10-mer oligonucleotide of the array does not differ in sequence from other adjacent 10-mer oligonucleotides, when aligned to each other, by at least 25% of the nucleotides (*id.* at ¶ 27). In fact, following the method of oligonucleotide synthesis taught by Brennan, each "oligonucleotide element, moving in a 5'-3' direction, is *identical* to the preceding element in nucleotide sequence except that it deletes the 5'-most nucleotide and adds a 3'-most oligonucleotide" (*id.*). Therefore, adjacent oligonucleotides formed according to the method of Brennan have significant sequence similarity when aligned (*id.*). In other words, nine out of ten nucleotides of adjacent 10mers are the same, so that adjacent 10mers have 90% sequence identity when aligned, differing by only 10% (*id.*).

Further, the PTO believes that the 10-mers of Brennan, representing all possible permutations of the 10-mer sequence would encompass the nucleotide multimers of the present patent application that differ by at least two nucleotides. However, because one 10-mer of Brennan will differ from a subsequently generated 10-mer by only one nucleotide, not all 10-mers of Brennan differ by at least two nucleotides. In addition, Brennan fails to teach that no two dimers within a 10-mer oligonucleotide are complementary to each other, and that the 10-mer oligonucleotides do not self-pair or undergo hairpin formation.

Froehler discloses oligomers containing 7-deaza-7-substituted purines and related analogs that have enhanced ability for double-and triple-helix formation with single- or double-stranded target nucleic acid sequences (Barany Declaration at ¶ 28). Such oligomer analog compositions can be used for diagnostic assays that employ methods where the oligomer or nucleic acid to be detected is covalently attached to a solid support (*id.*).

Froehler teaches that oligomers (*e.g.*, dimers – hexamers) are useful as synthons (*i.e.*, structural unit within a unit) for producing longer oligomers (*id.* at ¶ 29). However, Froehler fails to teach oligomers on a solid support where each oligomer differs in sequence from other adjacent oligomers, when aligned to each other by at least 25% of the nucleotides (*id.*).

Froehler also fails to teach that no two dimers within a synthon are complementary to each other and the synthons would not undergo self pairing or hairpin formation.

The combination of Fodor, Brennan, and Froehler does not teach arrays of oligonucleotides on a solid support where each capture oligonucleotide differs in sequence from other adjacent capture oligonucleotides, when aligned to each other by at least 25% and hybridize to complementary oligonucleotide target sequences under *uniform* hybridization conditions across the array of oligonucleotides (Barany Declaration at ¶ 30). In addition, the combination of Fodor, Brennan, and Froehler fails to teach selecting nucleotide multimers where no two dimers forming a nucleotide multimer are complementary to each other, and the nucleotide multimers would not result in self-pairing or hairpin formation.

Applicants' Array Design avoids all of the aforementioned problems associated with typical hybridization arrays (*i.e.*, target-capture probe cross-hybridization and false-positive/negative signal generation) (*id.* at ¶ 33). Identifying one or more target nucleotide sequences using Applicants' Array Design may employ a ligase detection reaction (LDR) followed by a high-throughput method of detection to decouple mutation discrimination from hybridization and detection (*id.*). Hybridization is carried out using divergent probe sequences that are not homologous to the target sequence being detected or

any other known genomic sequence (*id.*). Although divergent in sequence, these probes are carefully designed to have very similar hybridization properties (*id.*). This strategy significantly reduces cross-hybridization to enhance the specificity of target discrimination, while allowing for the use of uniform hybridization conditions across the array to facilitate a high-throughput assay format (*id.*).

A schematic representation of one embodiment of the invention using Applicants' Array Design to detect single base changes in a gene is provided in Figure 13 below (*id.* at ¶ 34).

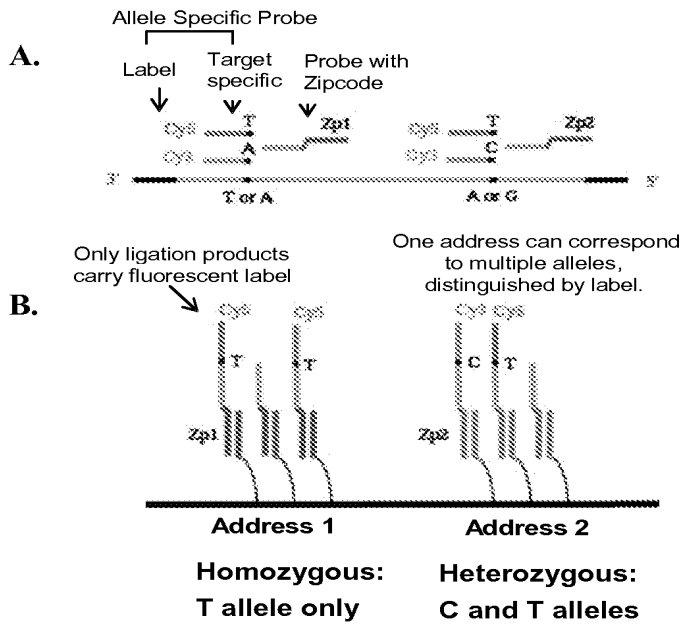

Figure 13: (A) LDR is performed using a common probe for each genetic locus which contains a unique addressable array-specific portion (Zp1 or Zp2) and allele-specific probes, each containing a unique detectable reporter label (*e.g.*, Cy3 or Cy5). Ligation of the two probes occurs only when there is perfect complementarity at the junction. (B) The multiplexed ligation products are captured on an addressable array containing capture probes that are complementary to the addressable array-specific portion of the common LDR probe.

As illustrated in Figure 13A, a plurality of oligonucleotide probe sets are used, where each probe set is characterized by (a) a first oligonucleotide probe having a target specific portion and an addressable array specific portion (Zp1 or Zp2) that is distinct from the target sequence and different for each gene locus that is interrogated, and (b) a second oligonucleotide probe having an allele-specific target portion and a unique detectable reporter label portion (*e.g.*, Cy5 or Cy3) (*id.* at ¶ 35). In an LDR process, the oligonucleotide probes are complementary to only one strand of the target nucleic acid as shown above, resulting in the linear amplification of the target nucleotide sequence (*id.*).

When oligonucleotide probes of a probe set hybridize adjacent to one another on a target sequence, ligation occurs only if there is perfect complementarity at the ligation junction (*id.* at ¶ 36). The resulting ligation product contains (a) the addressable array specific portion, (b) the target-specific portions, and (c) the detectable reporter label (*id.*). The addressable array-specific portion of a ligation product is complementary to a capture oligonucleotide immobilized at a particular site or "address" on the solid support (*id.*). As depicted in Figure 13B above, the addressable array-specific sequences together with a detectable label can discriminate between a plurality of different target sequences (*id.*).

The plurality of capture oligonucleotides immobilized on a solid support are designed to differ substantially from each other in their nucleotide sequence, yet all have the same or similar melting temperature (*id.* at ¶ 37). This design strategy drastically minimizes any chance of cross-hybridization leading to false-positive signals, while allowing for simultaneous capture of a plurality ligation products, by their addressable array sequence, under uniform hybridization conditions across the array (*id.*). In accordance with Applicants' Array Design, 24-mer capture oligonucleotides were designed that differed from each other by at least 6 bases or at least 25% when aligned to each other based on sequence similarities (*see* Figure 14 below), yet have the same or very similar melting temperatures (Tm) (*id.*).

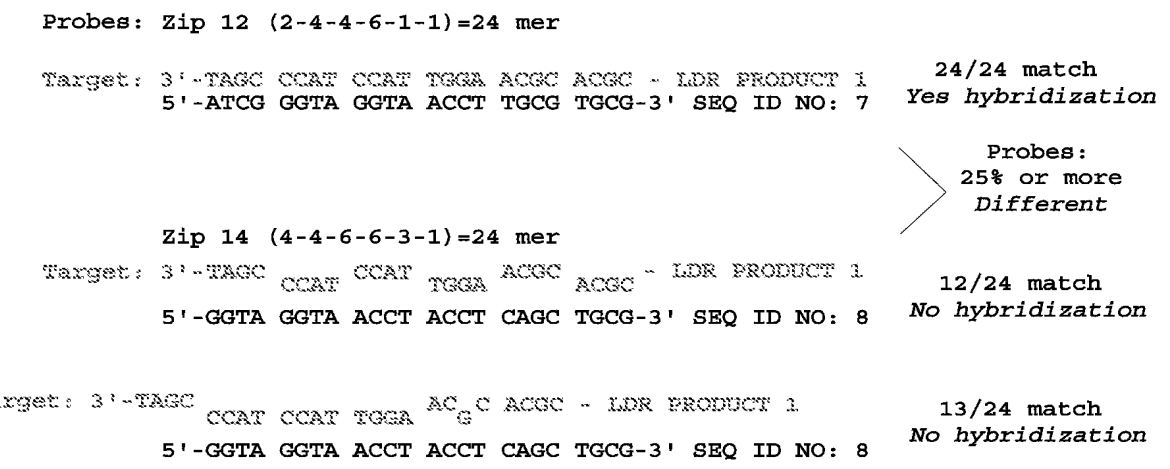

Figure 14. Capture oligonucleotides of the present invention are designed to differ from each other by at least 25% of their nucleotide sequence when aligned. Using this design strategy cross-hybridization between non-complementary addressable array portions and capture oligonucleotides will not occur.

As illustrated in Figure 14, cross-hybridization between an addressable array sequence and the wrong capture oligonucleotide probe sequence will not occur because of the extent of non-complementarity that exists between them (*id.* at ¶ 38). Because the capture oligonucleotide sequences remain constant (*i.e.*, the sequence is not target-specific), and their

complements can be appended to any set of LDR primers, the addressable arrays of Applicants' Array Design have universal application (*id.*).

As summarized above, Applicants' Array Design provides for the highly sensitive and specific detection and discrimination of target sequences that differ by only a single nucleotide substitution, deletion, or insertion in a sample (*id.* at ¶ 39). As summarized below and described in detail in the attached peer-reviewed publications, Applicants' Array Design provides a rapid and reliable method for the detection of genomic mutations (*e.g.*, genetic disease mutations and cancer related mutations), promoter methylation, and infectious diseases *(e.g.*, bacterial, fungal and viral infections (*id.*).

Gerry et al., "Universal DNA Microarray Method for Multiplex Detection of Low Abundance Point Mutations," *J. Mol. Biol.* 292:251–62 (1999) ("Gerry") demonstrates the simultaneous detection of seven of the most common point mutations in the K-*ras* gene that are involved in colorectal cancer using Applicants' Array Design coupled to an LDR assay (*id.* at ¶ 40). LDR probe sets comprising an allele-specific probe with an addressable array portion (also referred to as "zip code") and a common probe having a fluorescent reporter label were designed to detect the seven mutations in nine individual DNA samples obtained from cell lines or paraffin-embedded tumor tissue (*id.*). Following LDR, the ligated, fluorescently labeled LDR products were hybridized to an addressable DNA array containing capture oligonucleotides complementary to the addressable array sequences of the LDR products (*id.*).

Using this method all K-*ras* mutations in the tumor and cell line DNA were correctly identified without the generation of false-positive or negative signals (*id.* at ¶ 41). To determine the limit of detection of low level mutations in wild-type DNA (*i.e.*, assay sensitivity), mutant DNA was diluted in wild-type DNA in ratios ranging from 1:20 to 1:500 (*id.*). As shown in Figure 5 of Gerry, positive hybridization signal was quantifiable at a dilution of 1:200 with a signal-to-noise ratio of 2:1 (*id.*). These results confirmed the utility of Applicants' Array Design for detecting multiple nucleotide polymorphisms that are present in less than 1% of the total DNA (*id.*). Subsequently, a polymer flow-through biochip assembly that consists of a continuous-flow LDR microchip and a microarray chip has been fabricated that is capable of detecting one K-*ras* mutant sequence in the presence of 100 normal sequences (Hashimoto et al., "Ligase Detection Reaction/Hybridization Assays Using Three-Dimensional Microfluidic Networks for the Detection of Low Abundant DNA Point Mutations," *Anal Chem* 77:3243–3255 at abstract (2005) (*id.*).

In addition to single nucleotide substitution mutations, many cancers involve small nucleotide insertions and deletions which result in frameshift mutations (*id.* at ¶ 42). For example, a number of small insertions and deletions are found within the BRCA1 and BRCA2 genes that are associated with inherited breast and ovarian cancer (*id.*). A number of these insertion and deletion mutations are refractory to detection by direct hybridization array approaches, requiring the development of an alternative method (*id.*). As described below, Applicants' technology is sensitive enough to detect sporadic mutations directly from tumor tissue within the p53 gene, which is involved in nearly half of all human cancers (*id.*).

Favis et al., "Universal DNA Array Detection of Small Insertions and Deletion in BRCA1 and BRCA2," *Nat. Biotech.* 18:561–564 (2000) ("Favis"), demonstrates the capacity of Applicants' Array Design to reliably and reproducibly detect small nucleotide insertions and deletions using the BRCA1 and BRCA2 genes as a model system (*id.* at ¶ 43). As shown in Figure 1 of Favis, the method of detecting insertion and deletion mutations coupled a multiplex PCR step to LDR and Applicants' Array Design (*id.*). This approach reproducibly detected both insertion and deletion mutations in BRCA1 and BRCA2 (*i.e.*, BRCA1 185delAG; BRCA1 5382insC; and BRCA2 6174delT) (*id.*). No cross-hybridization was detected, supporting the specificity of the method, and the reproducibility of the results were confirmed using a gel-based method (*id.*). Further, even the presence of mutations in pooled samples was detected (*id.*).

p53 mutations are observed in approximately one-half of all human cancers (*id.* at ¶ 44). Applicants' Array Design, when applied to the detection of p53 mutational status of clinical biopsy samples containing <5% tumor cells, was able to detect all mutations that were detected by direct sequencing and a yeast functional assay (Fouquet et al., "Rapid and Sensitive p53 Alteration Analysis in Biopsies from Lung Cancer Patients Using a Functional Assay and A Universal Oligonucleotide Array: A Prospective Study," *Clin Cancer Res* 10:3479–3489 at abstract and p. 3483, col. 2, para. 2 (2004) (*id.*). This approach was also used to detect 58 different p53 mutations in undissected colon tumor DNA samples (Favis et al., "Harmonized Microarray/Mutation Scanning Analysis of TP53 Mutations in Undissected Colorectal Tumors," *Human Mutation* 24:63–75 (2004)) (*id.*).

An important feature of Applicants' Array Design is that it is not one-dimensional in it diagnostic utility (*id.* at ¶ 45). In addition to being a highly sensitive and robust method for detecting single base substitutions, insertions, and deletions involved in cancer development and progression, the method of coupling LDR to Applicants' Array

Design has been successfully applied to the determination of promoter methylation status (Cheng et al., "Multiplexed Profiling of Candidate Genes for CpG Island Methylation Status Using a Flexible PCR/LDR/Universal Array Assay," *Genome Research* 16(2):282–9 at abstract (2006) (*id.*). DNA methylation in CpG islands is associated with transcriptional silencing, and the ability to accurately determine cytosine methylation status in promoter CpG dinucleotides provides diagnostic and prognostic value for many human cancers (*id.*). Applicants' Array Design demonstrated the ability to clearly distinguish different levels of methylation at 75 independent CpG dinucleotides in the promoter regions of 15 tumor suppressor genes (*id.*). When compared with an independent pyrosequencing method at a single promoter, the two approaches gave good correlation (*id.*). In a study using 15 promoter regions and seven blinded tumor cell lines, Applicants' technology was capable of distinguishing methylation profiles that identified cancer cell lines derived from the same origins (*id.*). Further, Applicants' approach has the sensitivity required to detect the presence of methylation at 0.5% without selective PCR amplification, and at 0.05% with methyl-specific PCR amplification (*id.*). This would correspond to identifying one tumor cell in 200 normal cells, or one tumor cell in 2,000 normal cells, respectively (*id.*). This level of sensitivity holds the promise for early detection of colon cancer in DNA isolated from stool or serum. (*id.*).

Applicants' Array Design can be utilized for identifying and distinguishing infectious agents (*e.g.*, bacterial, viral, and fungal) in many areas of biomedical science, including health care, biological defense, and environmental monitoring (*id.* at ¶ 46). The detection and identification of infectious agents must be highly sensitive and specific to distinguish closely related species or serotypes whose genomic sequences in specific regions differ at only a few nucleotide positions (*id.*).

Das et al., "Detection and Serotyping of Dengue Virus in Serum Samples by Multiplex Reverse Transcriptase PCR-Ligase Detection Reaction Assay," *J. Clin. Microbiol.* 46(10):3276–84 (2008) ("Das") demonstrates the simultaneous serotyping and genotyping of dengue virus (DENV) in viral cultures and patient samples by coupling PCR based amplification to LDR and Applicants' Array Design (*id.* at ¶ 47). The assay accurately identified and serotyped DENV in 350 archived acute-phase serum samples, demonstrating 98.7% sensitivity and 98.4% specificity for detection (*id.*). The detection limit for the assay ranged from 0.004 to 0.7 plaque forming units (PFU)/ reaction, comparable to those reported for other techniques (*id.*). The assay was highly specific for the detection of DENV with no

cross reactivity to seven other similar flavivirus (*id.*). This assay has also been employed for the successful identification of West Nile viral strains, which also exhibit considerable genomic diversity, in clinical samples (*see* Rondini et al, "Development of Multiplex PCR-Ligase Detection Reaction Assay for Detection of West Nile Virus," *J. Clin. Microbiol.* 46:2269–79 (2008)) (*id.*).

There are numerous advantages afforded by Applicants' Array Design (*id.* at ¶ 48). Direct hybridization arrays were designed to detect single nucleotide polymorphisms, with discrimination based on hybridization of target sequences to perfectly matched or mismatched probes (*id.*). However, as discussed *supra*, the utility of direct hybridization methods to accurately and reproducibly detect and discriminate even these single nucleotide variations is questionable (*id.*). The extent of sequence similarity between mismatch and perfect match probe sequences enables cross-hybridization and leads to the generation of both false-positive and false-negative signals (*id.*). The findings of Naiser and Pozhitkov suggest that the problem of cross-hybridization is only further compounded by the unpredictable nature of oligonucleotide probe-target hybridization (*id.*). Direct hybridization methods are not well suited for the detection of most other types of nucleic acid sequence variations (*id.*). In fact, in most cases, the ability to detect insertion/deletion mutations has proven intractable (*id.*).

In contrast to direct hybridization methods, Applicants' Array Design has demonstrated the ability to detect insertion/deletion mutations, mononucleotide and dinucleotide repeats, and even methylation of CpG islands (*id.* at ¶ 49). In addition, it can be used to identify and quantify splice site changes, quantify RNA levels for gene expression profiling, and determine DNA copy levels changes, loss of heterozygosity, and SNPs for genome-wide association studies (*id.*).

A significant advantage of the Applicants' Array Design is that it relies on divergent capture-specific probe sequences, designed to differ in sequence by at least 25%, yet have similar melting temperatures (*id.* at ¶ 50). The result: cross-hybridization is minimized, if not eliminated, even under uniform hybridization conditions (*id.*). The composite probes and products, which contain a target specific portion and a capture specific portion allow for accurate target identification and discrimination of closely spaced and overlapping mutations, including small insertions and deletions without generating false-positive or false-negative signals (*id.*). In addition, the method has proven to be highly sensitive, capable of detecting low abundance mutations in heterogenous clinical samples

(*id.*). This sensitivity permits early disease detection, which can be critical for a good disease prognosis (*id.*). The ability to use the method to detect promoter methylation silencing of tumor suppressor genes helps predict disease outcome and guide cancer treatment (*id.*).

Applicants respectfully submit that the claimed invention is neither taught by, nor rendered obvious in view of the combination of the Fodor, Brennan, and Froehler. In addition, the claimed invention has successfully resolved an unmet and long-felt need in the art for an assay that can achieve early and accurate detection of cancer related genomic mutations and infectious diseases. Accordingly, the rejection of claims 89–97, 109, 111–112, and 148–153 based on these references is improper and should be withdrawn.

The rejection of claims 89–94, 96–97, 109, 111–112, and 148–153 under 35 U.S.C. § 103(a) for obviousness over U.S. Patent No. 5,527,681 to Holmes ("Holmes") in view of Brennan and Froehler is respectfully traversed for the reasons set forth in the accompanying Barany Declaration.

Holmes relates to methods, devices, and compositions for synthesis and use of diverse molecular sequences on a substrate (Barany Declaration at ¶ 31). In particular, Holmes discloses the synthesis of an array of polymers in which individual monomers in a lead polymer are systematically substituted with monomers from one or more basic sets of monomers (*id.*). On the substrate or a distal end of linker molecules, a functional group with a protective group is provided (*id.*). The protective group may be removed upon exposure to a chemical reagent, radiation, electric fields, electric currents, or other activators to expose the functional group (*id.*). Concurrently or after exposure of a known region of the substrate to light, the surface is contacted with a first monomer unit M1 which reacts with the functional group which has been exposed by the deprotection step (*id.*). Thereafter, second regions of the surface (which may include the first region) are exposed to light and contacted with a second monomer M2 (which may or may not be the same as M1) having a protective group (*id.*). These steps are repeated until the substrate includes desired polymers of desired lengths (*id.*). Monomers may include amino acids, nucleotides, pentoses, and hexoses (*id.*).

Holmes, like Brennan and Froehler, does not teach arrays of oligonucleotides on a solid support where each oligonucleotide of the array differs in sequence from other adjacent oligonucleotides, when aligned to each other by at least 25% (Barany Declaration at ¶ 32). Further, the combination of Holmes, Brennan, and Froehler fails to teach a method that such oligonucleotides are attached to a solid support and hybridize to complementary oligonucleotide target sequences under *uniform* hybridization conditions across the array of oligonucleotides (*id.*).

Further, Holmes does not teach that each nucleotide multimer has a nucleotide sequence that differs from the nucleotide sequence of another selected nucleotide multimers by at least two nucleotides, and where no two dimers within a nucleotide multimer are complementary to each other. Holmes also fails to teach that the multimers would not result in self-pairing or hairpin formation.

Accordingly, the rejection of claims 89–94, 96–97, 109, 111–112, and 148–153 under 35 U.S.C. § 103(a) for obviousness over Holmes in view of Brennan and Froehler is improper and should be withdrawn.

The rejection of claims 89 and 149-151 under the judicially-created doctrine of obviousness-type double patenting over claims 1 and 2 of U.S. Patent Application Serial No. 10/257,158, now U.S. Patent No. 7,455,965 ("'965 patent"), is respectfully traversed.

The '965 patent relates to a method of designing a plurality of capture oliogonucleotide probes, to which complementary oligonucleotide probes will hybridize. This method involves providing a first set of tetramers, linking groups of 2 to 4 of the tetramers from the first set together to form a collection of multimer units, and then removing from the collection various multimer units to form a modified collection of multimer units. The modified collection of multimers are randomized, divided into first and second sub-collections, and arranged according to melting temperature. Multimers of the first sub-collection are then linked to multimers of an inverted second sub-collection to form a collection of double multimer units which is subsequently further manipulated to produce a modified collection of double multimer units.

The claimed invention is patentably distinct from that of the '965 patent, because the claims of each do not disclose the specifics of the other. In particular, the claims of the present invention do not recite calculating the predicted melting temperatures of multimer units in the collection of multimer units, and removing from the collection all multimers units formed from the same tetramer and all multimer units having a melting temperature in ^{o}C of less than 4 times the number of tetramers forming a multimer unit, to form a modified collection of multimer units, as recited by the claims of the '965 patent. In addition, the claims of the present invention do not recite arranging the modified collection of multimer units into groups differing by 2^{o}C increments in melting temperature, randomizing the order of the groups of multimer units, dividing alternating groups of multimer units into first and second subcollections, arranging each of the first and second subcollections in order of melting temperature, inverting the order of the second subcollection, and linking the multimer units of the first subcollection to the multimer units of the inverted second

subcollection, to form a collection of double multimer units. Finally, the claims of the present invention do not recite calculating the melting temperature of the linked multimer units in the collection of double multimer units, removing from the collection of double multimer units all double multimer units (1) having a melting temperature in °C of less than 11 times the number of tetramers and more than 15 times the number of tetramers, (2) double multimer units with the same 3 tetramers linked together, and (3) double multimer units with the same 4 tetramers linked together with or without interruption, to form a modified collection of double multimer units, as recited by the claims of the '965 patent.

Likewise, the claims of the '965 patent do not recite assembling nucleotide multimers as capture oligonucleotides where the nucleotide multimers are selected so that each of the plurality of capture oligonucleotides formed from a plurality of assembled nucleotide multimers and attached to the solid support at each array position, have greater than sixteen nucleotides and have nucleotide sequences selected to hybridize with complementary oligonucleotides target sequences under uniform hybridization conditions across the array of oligonucleotides with minimal cross-reactivity, as recited by the claims of the present application. In addition, the claims of the '965 application do not recite that each capture oligonucleotide of the array differs in sequence from other adjacent capture oligonucleotides, when aligned to each other by at least 25% of the nucleotides.

In view of the substantial differences between the claims of the present application and the claims of the '965 patent, the double patenting rejection based on the '965 patent is improper and should be withdrawn.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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